THE RELATION OF THE CHARACTERISTICS AND YIELD OF WAX TO PLANT AGE

E. B. KURTZ, JR.[1]

(WITH TWO FIGURES)

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Introduction

Plants have been used as a source of wax for centuries, and the search for plant species that produce more wax of a better quality has been in progress for as many years. It has been recognized only for the past few decades, however, that various factors other than plant species, such as environment, genetic make-up of the plant, and maturity of the plant, may affect the quality and quantity of wax exudate. McNAIR (**11**) has shown that there is a definite relation between the properties of plant waxes and climate; and CONRAD and NEELY (**2**) in a study of cotton showed that an hereditary factor controls the quantity of lint wax. Only fragmentary evidence, however, has been presented to show that plant maturity may affect the deposition of wax on the plant surfaces. HOWES (**5**) observed that the yield of wax from the carnauba palm, candelilla, and fruits of bay-berry varied in some way with age of the plant organs. DAHLGREN (**3**) studied the yield of carnauba wax from unexpanded and expanded leaves and concluded that under some conditions expanded or old leaves yield more wax per leaf than unexpanded or young leaves. Studies by SAHAI and CHIBNALL (**13**) on the wax of Brussels sprout, and JORDAN and CHIBNALL (**8**) on the wax of the runner bean, have shown that wax is synthesized continually throughout the life of the plant, with the formation of wax occurring not just at the growing tips but in all the plant organs except the roots. It is apparent, therefore, that the synthesis and yield of wax are related to plant age, and that the relationship might vary greatly in some cases. It has been the purpose of the present study to observe the relation of the yield and the physical and chemical characteristics of wax to plant maturity.

Materials and methods

On the basis of time of collection and external structural characteristics, two ages (young and old) of leaves or stems of *Phragmites communis* (Gramineae); *Acacia greggii, Acacia vernicosa, Acacia constricta, Olneya tesota* (Leguminosae); *Asclepias linaria* (Asclepiadaceae); *Beloperone californica* (Acanthaceae); staminate and pistillate plants of *Baccharis sarothroides, Aplopappus tenuisectus,* and *Aplopappus laricifolius* (Compositae) were collected for study from their natural habitats. These species

[1] Present address, Kerckhoff Laboratories of Biology, California Institute of Technology, Pasadena, California.

are all indigenous to southern Arizona, and are representative of wide ranges of environmental conditions of altitude, rainfall, incident light, and edaphic factors. Several ages of leaves of *Sorghum halepense* (Gramineae) and pistillate *Phoenix dactylifera* (Palmaceae) were also studied. Eight ages of plant material of *S. halepense* were distinguished by their position on the culm. The inflorescence and seven ages of leaves and nodes were collected separately, progressing from the inflorescence and youngest node and leaf at the top to the oldest node and leaf at the base of the culm. The time interval between each age of node and leaf was approximately five days. From their position on the tree, leaves of *Phoenix dactylifera* of 0, 3, 12, 18, 24, and 36 months of age were distinguished for study.

Several pounds of each age of plant material were collected and air-dried. The residual moisture after drying was 5% or less. A modification of the method of CHIBNALL *et al.* (**1**) was used to extract and separate fractions referred to as the wax and non-wax fractions. The air-dried plant material was refluxed for two hours with petroleum ether (B.P. 30° to 60° C). Decantation of the extract followed by several rinses of the plant material with fresh hot solvent completed the extraction. The extract was reduced to 250 ml. by distillation of the excess solvent. The wax was precipitated as an acetone-insoluble fraction by the addition of 500 ml. of acetone. The acetone-soluble fatty substances, or non-wax fraction, were recovered by evaporation of the solvent after removal by filtration of the so-called wax fraction. The per cent. yield of both wax and non-wax fractions was calculated on the basis of the weight of the dry plant material extracted.

The wax fractions were characterized physically by the determination of the drop point melting point (**12**), with the exception of a few cases in which the capillary rod drop melting point (**9**) was determined because only minute quantities of the wax samples were available. Relative hardness, color, and odor of the waxes were also noted.

Chemical characterization of the wax and non-wax fractions was accomplished by determining the acid, saponification, ester, and Wijs iodine numbers (**6**). These tests give, respectively, the amount of free acid, saponifiable matter, and esters present in, and the degree of unsaturation of the lipid fractions from the different ages of plant material.

At the beginning of the study an attempt was made to correlate the variations of wax yield and characteristics with changes in the carbohydrate content of the different ages of plant. Determination by the method of HASSID (**4**) of the milligrams per gram of the different ages of leaves and nodes of *Sorghum halepense* of total sugars, reducing sugars, sucrose, and starches and dextrins failed to yield any data of apparent significance. This line of study was not continued.

Because the wax yielded from a plant is generally believed to be derived from the cuticle, the relationship between wax yield and cuticle thickness was studied. A small sample of each age of plant material studied was killed and fixed in formalin-aceto-alcohol and then run up through the tertiary butyl alcohol and paraffin series according to JOHANSEN (**7**). After sectioning at ten microns and staining, the cuticle thickness was measured in microns.

Results and discussion

The relationship of wax yield to plant age varied with different species, and on a weight per cent. basis increased in some cases and decreased in others. Nevertheless, in most cases (with the exception of *Acacia greggii, Acacia constricta, Aplopappus laricifolius,* and *Phoenix dactylifera*) this yield change with age was found to be directly related to cuticle thickness (tables I and II). This indicates that the main source of wax is the cuticle, and that only minor amounts of wax would be found in the protoplasm. According to earlier studies on Brussels sprout (**13**) and the runner bean (**8**), wax is steadily synthesized as the plant matures and is not catabolized. If this behavior is considered general, then the decrease with age of wax yield in several cases of the present study was only an apparent decrease. It is probable that by abrasive action of rain, wind, and dust on the cuticle, or by flaking and sloughing of the cuticle as the plant matured, the cuticle in these cases decreased in thickness and therefore the yield of wax decreased. Very marked increase of the dry weight of the plants with age may also have caused an apparent decrease of wax yield. In general it may be concluded that the total wax content of a plant increases with plant maturity; and in species requiring many years to mature, wide variation of yield may be found between young and old parts.

The yields of the non-wax extracts also show considerable variance with age, but there is no indication from yields that wax was synthesized at the expense of the non-waxes.

The results in tables I and II and figures 1 and 2 show that the wax melting point usually remained unchanged or it increased slightly with age. It also will be noted that the iodine number of the wax fraction usually decreased with age. A comparison of the two characteristics shows that in *Acacia greggii, Acacia constricta, Beloperone californica, Asclepias linaria,* and *Sorghum halepense* an increase of the melting point with age was associated with a decrease of the iodine number of the same wax. This relationship is in agreement with MARKLEY (**10**) that the melting point of a lipid substance may be raised by a decrease in unsaturation. The decrease in unsaturation of the waxes with age may have been brought about by oxidation of the wax compounds upon prolonged exposure to the atmosphere.

In the case of both *Sorghum halepense* and *Phoenix dactylifera,* the iodine number of the non-waxes increased through several stages of the early growth. However, in the most mature leaves the degree of unsatura-

TABLE I

EXTRACT YIELDS, CUTICLE THICKNESS, AND CHARACTERISTICS OF THE WAX AND NON-WAX FRACTIONS OF ELEVEN SPECIES BY AGE OF THE PLANT

SPECIES	AGE	CUTICLE THICKNESS	WAX					NON-WAX		
			YIELD	M.P.	IODINE no.	ACID no.	ESTER no.	YIELD	ACID no.	ESTER no.
		μ	%	°C				%		
Phragmites communis	young	2.6	0.16	80.0	1.17	9.41	52.5	0.20	45.23	150.5
	old	1.75	0.05	80.0	0.79	26.00	57.2	0.22	29.35	218.0
Acacia greggii	young	2.27	0.10	77.0	5.19	11.29	53.3	0.15	22.19	0.0
	old	2.2	0.32	79.5	0.39	6.05	75.0	0.14	27.83	111.7
Acacia vernicosa	young	....	0.15	77.0	2.65	5.04	69.6	0.16	70.58	65.6
	old	....	0.30	75.0	2.26	15.25	73.4	0.15	72.36	172.4
Acacia constricta	young	2.27	0.41	71.0	5.65	4.11	81.5	0.29	22.45	109.3
	old	2.27	0.30	75.0	1.11	9.06	79.2	0.20	18.82	3.6
Olneya tesota	young	2.3	0.30	74.0	1.26	2.08	24.4	0.20	15.95	54.2
	old	3.05	0.38	73.0	1.16	6.91	46.5	0.23	12.93	143.6
Asclepias linaria	young	1.75	1.26	67.0	73.06	2.43	10.4	1.79	9.25	54.2
	old	1.5	0.30	70.0	13.12	11.25	13.5	0.60	8.09	37.3
Beloperone californica	young	trace	0.37	70.0	13.02	5.35	16.6	0.22	19.22	66.2
	old	0.0	0.07	71.0	8.62	14.77	9.1	0.07	16.39	171.8
Baccharis sarothroides ♀	young	5.25	0.24	83.5	25.02	60.24	61.9	0.44	47.73	115.5
	old	4.5	0.15	80.0	17.04	52.70	36.6	0.42	38.89	93.0
Baccharis sarothroides ♂	young	3.5	0.06	78.3*	39.02	15.36	33.3	0.47	67.70	0.0
	old	5.25	0.21	78.5	53.85	14.25	52.4	0.57	33.81	4.5
Aplopappus tenuisectus	young	3.5	0.11	72.0	35.41	4.93	20.1	0.54	70.31	0.0
	old	4.8	0.34	72.0	23.97	12.07	15.2	0.56	59.96	0.0
Aplopappus laricifolius	young	1.75	0.06	66.5	29.61	14.92	0.0	0.48	60.83	24.1
	old	1.75	0.15	66.5	9.68	34.61	0.0	0.28	73.66	46.9

* Determined by capillary rod method

tion rapidly decreased in *S. halepense* and increased rapidly in *P. dactylifera*. The increase with age of unsaturation of the fatty constituents is in agreement with previous work (**8, 13**), but the significance of the rapid decrease of the iodine number with age of *S. halepense* is not known. It may be that as the old plant parts become desiccated, the unsaturated lipid compounds of this species are oxidized more rapidly by atmospheric oxygen.

The results of acid number determinations show that the amount of free acids in the wax fraction of some species decreased rapidly in the very young plant growth, and then increased during later growth. The acid content from the older material did not reach the amount found in the wax from the youngest leaves (figs. 1 and 2). These results may be correlated

TABLE II

EXTRACT YIELDS AND THICKNESS OF THE CUTICLE BY AGE OF *Sorghum halepense* AND *Phoenix dactylifera*

SPECIES	AGE	CUTICLE THICKNESS	YIELD	
			WAX	NON-WAX
		μ	%	%
	tops	....	trace	0.33
	5 days	3.50	0.04	0.55
	10 days	3.50	0.05	0.58
Sorghum	15 days	3.67	0.06	0.56
halepense	20 days	3.60	0.05	0.34
	25 days	....	0.05	0.31
	30 days	....	0.05	0.25
	35 days	....	0.04	0.19
	0 months	1.75	0.17	0.19
	3 months	2.60	0.15	0.16
Phoenix	12 months	3.50	0.10	0.15
dactylifera	18 months	3.50	0.19	0.13
	24 months	3.00	0.15	0.15
	36 months	3.00	0.15	0.24

with those of table I. The young plant material of *Acacia greggii* and pistillate and staminate plants of *Baccharis sarothroides* were collected in a very young condition. The wax acid number from the youngest material was higher than that from the old material. Therefore the over-all trend appears as a decrease of acid number with age. This is in agreement with the over-all trend of the acid number of the wax fractions of *Sorghum halepense* and *Phoenix dactylifera*. The young plant material of the other species of table I was not collected in a very young stage of growth, so that the over-all trend in acid number with age of these species was an increase. This increase corresponds to the latter part of the acid number curves of *S. halepense* and *P. dactylifera*. Thus it may be said that without exception, in the species studied, the wax from very young leaves had a high acid content; that there was a rapid decrease during immediate growth there-after; and that only during later periods of growth did the acid content of the wax increase. The significance of the rapid drop of wax acids in young leaves is not clear, but it may be associated with the synthesis of wax esters, as shown in figures 1 and 2.

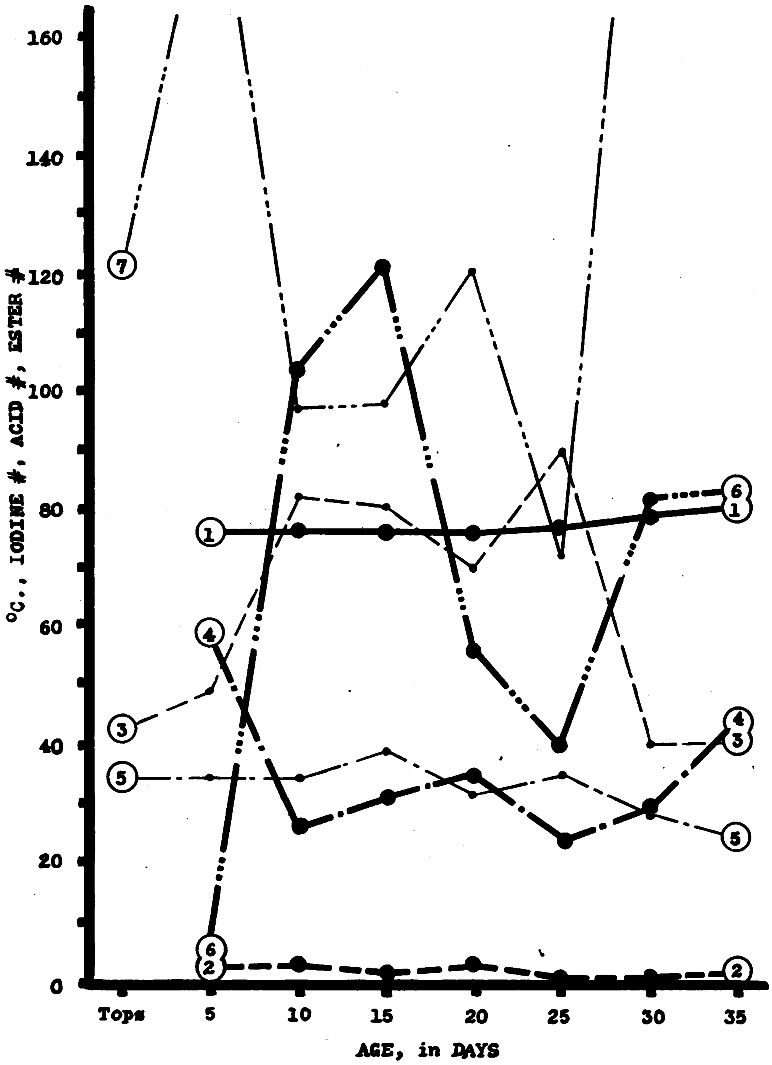

FIG. 1. Variations with age of *Sorghum halepense* of the characteristics of the wax and non-wax fractions (Wax: 1-1 melting point, 2-2 iodine no., 4-4 acid no., 6-6 ester no.; Non-wax: 3-3 iodine no., 5-5 acid no., 7-7 ester no.).

The free acids of the non-wax fraction appear to be related physiologically to the non-wax esters and the wax esters and acids. As has been discussed, the wax acid content increased with age after the very early stages of growth. The ester content of both wax and non-wax fractions also increased with age of the plant. The non-wax acid content, on the other hand, remained about the same or decreased with age of the plant. These results suggest that cellular fatty acids (the non-wax acids) possibly are precursors of the esters of both fractions and the acids of the wax fraction. This would account for the decrease of the non-wax acid number and the development of the characteristics of the other constituents with age. The

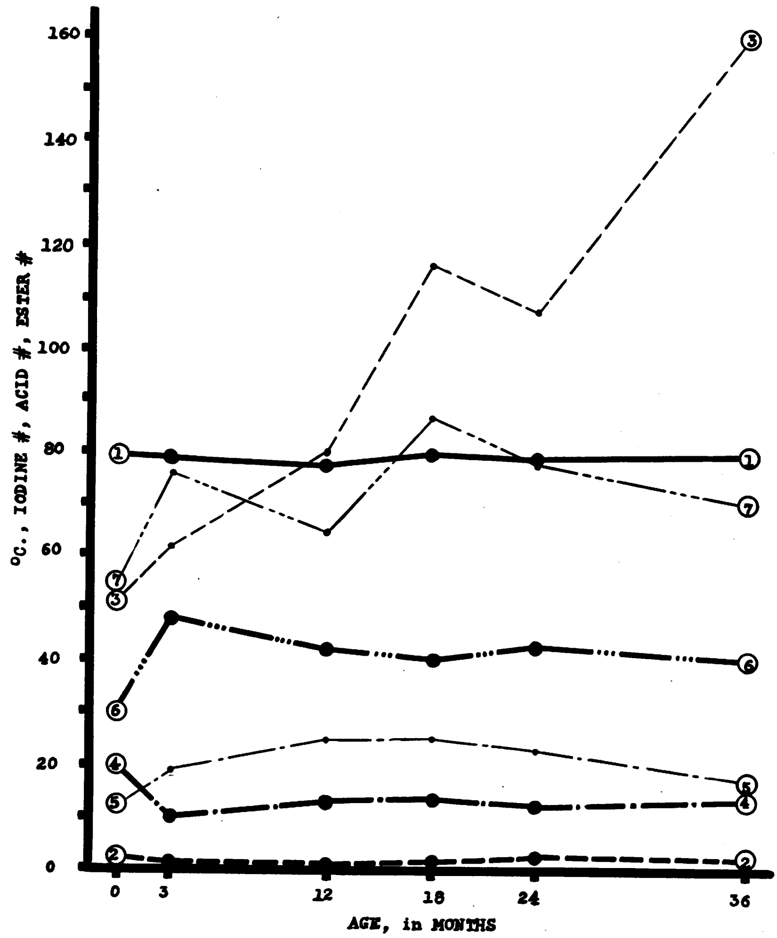

FIG. 2. Variations with age of *Phoenix dactylifera* of the characteristics of the wax and non-wax fractions (Wax: 1-1 melting point, 2-2 iodine no., 4-4 acid no., 6-6 ester no.; Non-wax: 3-3 iodine no., 5-5 acid no., 7-7 ester no.).

chemical reactions involved might be that cellular fatty acids condense to form higher molecular weight fatty acids that appear in the wax fraction. Reduction of the wax acids would form alcohols, and esterification would then form wax esters. Similar reactions could account for the increase of non-wax esters. The anabolism of the cellular fatty acids themselves, however, is a matter of conjecture, inasmuch as the only mechanisms which have been proposed have been largely theoretical.

Variations with age of the color and odor of the wax and non-wax fractions are readily interpreted. In general, the color and odor of the extracts corresponded to the pigmentation and odoriferous nature of the plant studied. No attempt will be made, however, to explain certain variations with age of hardness and brittleness of the wax fraction. Undoubtedly hardness and brittleness of a wax are associated with very complex inter- and intra-molecular factors which were not studied.

It will be noted from table I that the results from the study of staminate and pistillate plants of *Baccharis sarothroides* are frequently at variance with the general trends exhibited by the other species. The additional

TABLE III

THE WAX AND NON-WAX CHARACTERISTICS OF *Sorghum halepense* AND *Phoenix dactylifera* BY AGE OF THE PLANT

SPECIES	AGE OF LEAVES	WAX				NON-WAX		
		M.P.	IODINE NO.	ACID NO.	ESTER NO.	IODINE NO.	ACID NO.	ESTER NO.
		°C						
	tops	....	....	....	....	42.96	34.21	121.30
	5 days	76.5*	3.64	58.79	4.5	49.70	34.70	190.83
	10 days	76.5*	3.99	26.63	105.2	81.16	34.16	97.33
Sorghum	15 days	76.3*	1.66	31.89	123.9	80.53	38.37	99.02
halepense	20 days	76.3*	3.73	35.24	57.0	70.58	32.27	120.37
	25 days	76.8*	0.00	23.32	40.3	90.84	35.62	71.31
	30 days	78.5*	0.00	29.88	82.2	39.96	28.45	245.59
	35 days	79.8*	1.55	41.21	83.5	41.74	24.01	197.26
	0 months	80.0	2.19	20.17	30.0	51.12	12.52	54.12
	3 months	79.5	1.67	10.73	49.0	63.40	19.18	76.26
Phoenix	12 months	77.5	0.97	13.96	43.0	80.54	25.17	65.15
dactylifera	18 months	80.0	1.44	13.28	41.1	116.88	25.18	87.11
	24 months	78.5	2.44	12.41	43.0	107.01	22.93	78.60
	36 months	79.5	2.37	13.25	39.7	158.94	17.90	70.06

* Determined by the capillary rod method.

factor, sex, evidently showed precedence over the age factor, and therefore caused results at variance with those of the other species. The influence of sex on lipid metabolism apparently is quite strong. A study of the sexual relationships is being continued.

Summary

1. Thirteen species were studied as to the relationships of wax to plant age. After extraction with petroleum ether, the lipid extract was separated into wax and non-wax fractions. The yields and physical and chemical characteristics of each fraction were determined. All ages of plant material studied were also subjected to histological studies of the cuticle.

2. The yields of the wax and non-wax fractions by age varied differently with each species. The yield of the wax fraction varied directly with the thickness of the cuticle.

3. The wax melting point did not change markedly with age, but in some species there was a slight increase. This increase in melting point was correlated with a decrease in wax unsaturation. It was suggested that the decrease in unsaturation with age is caused by oxidation by atmospheric oxygen.

4. The degree of unsaturation of the non-wax fraction usually increased with age.

5. The amount of wax acids was found to decrease rapidly in young plants and then slowly increase as the plant matured. This variation of acid content appeared to be related to wax ester synthesis.

6. The amount of wax esters and acids and non-wax esters increased with age. It was considered that this might be correlated with the decrease or maintenance of the non-wax acid concentration as the plant matured. The cellular fatty acids appeared to be precursors of esters and wax fraction acids.

7. The color and odor of the wax and non-wax fractions were directly related to the type of pigmentation and odor of the plant.

8. No relationship was found between carbohydrate and lipid content.

9. The divergence from the general trends of the results of the staminate and pistillate plants of *Baccharis sarothroides* was believed to be associated with a sexual factor.

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DEPARTMENT OF BOTANY AND RANGE ECOLOGY
COLLEGE OF AGRICULTURE
UNIVERSITY OF ARIZONA
TUCSON, ARIZONA

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